

SEROLOGICAL AND MOLECULAR DETECTION OF ROTAVIRUS INFECTION IN CHILDREN UNDER FIVE YEARS IN NASSYRIAH PROVINCE

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ABSTRACT

The present study was conducted to investigate the prevalence of rotavirus (RV) disease among children under 5 years old whom consulting Bint-Al Huda teaching hospital in Nassyriah province, Iraq. Two hundred and fifty stool samples obtained from children with acute diarrhea was screened for RV infection using Enzyme linked Immunosorbant Assay (ELISA), as serological tool, and Real-Time quantitative Polymerase Chain Reaction (RT-qPCR), as molecular technique, during the period of November 2011 to June 2012.

Out of 250 stool samples, rotavirus infection was detected in 72 (28.8%) child by both technique. LISA test results showed that 54 were RV-positive sera with a percentage of (21.6%). RT-qPCR technique was used to detect the presence of rotavirus in 96-negative ELISA samples. The results showed that there were 18 stool samples showed RV-positive reaction for NSP4 gene with a percentage of infection as (18.75%). The most common clinical features included diarrhea with 72 (100%) child, in addition to, vomiting in 31 cases s (43.06%) and fever with 15 (20.83%).

Males tends to be more affected with RV infection with 40 (55.56%) child in compare to female with 32 (44.44%) child ($p>0.05$). The peak incidence of RV diarrhea was seen in children aged (1-7) and (8-15) months with a total RV-positive sera of 64 (88.89%) child ($p<0.01$). Among the study period, April recorded, statistically, the highest prevalence of RV-positive cases with 27 (37.50%) child ($p<0.01$). On the other hand, January showed the lowest RV-positive cases with 2 (3.70%) cases only. The results of the present study revealed that the rate of RV gastroenteritis was found to be highest in patients who were used breast feeding with 36 (50.0%) child, followed by bottle and mixed feeding with 25 (34.72%) and 11 (15.28%) children, respectively ($p<0.01$). RV patients consuming R.O drinking water supply had, significantly, higher infection rate with 36 (50.0%) child, in compares to those used either mineral or tap water with 23 (31.94%) and 13 (18.06%) child, respectively ($p<0.01$). According to the previous finding, we can conclude that rotavirus infection as a cause of a cute diarrhea, may be seems to be common in Thi-Qar province and is increasing in high manner.

KEYWORDS: Prevalence of Rotavirus (RV) Disease, Thi-Qar Province, Pediatric diarrhea

INTRODUCTION

Pediatric diarrhea remains one of the major causes of death among infants. This is especially true in Asia, Africa, and Latin America, where it causes millions of deaths in the 0–5 years age group. The immediate causes are often of an infectious nature and include a variety of pathogenic microorganisms. Several different groups of viruses are responsible for the high rate of acute viral diarrhea condition among children during their first years of life.

Human rotaviruses (HRVs) represent the main cause of gastroenteritis in children worldwide (Parashar et al., 2003). It is estimated that they are responsible for a large number of diarrhea associated hospitalizations in childhood each year (Parashar et al., 2006). These infective agents are ubiquitous and about 95% of children worldwide are

infected before 3–5 years of age (Parashar et al., 1998). During the first 12 months of life, the risk of severe forms of infection, which require hospitalization, is particularly high, due to the high risk of dehydration in infants and the difficulty in restoring their electrolyte balance. HRV infections can affect adults, often in sub-clinical forms, and occasionally determine clinically evident cases in immunodeficient patients, in the elderly, and in travelers who visit developing countries (Anderson and Weber, 2004).

HRVs are members of a distinct genus of the Reoviridae family. They are non-enveloped triple-layered particles, with a double capsid, a core containing the viral genome, and two surface proteins, VP4 and VP7. HRVs are classified into seven serogroups (A–G) on the basis of the antigenic properties of shared epitopes on the major structural protein, VP6 (Clark and McKendrick, 2004). Groups A–C are found in both humans and animals, whereas groups D–G have only been found in animals. Group A rotaviruses have clearly been established as causing significant diarrheal disease in infants and in the young of various mammalian and avian species. Within group A, rotaviruses are classified by a binary system into at least 23 G types (VP7) and 31 P types (VP4) (Schumann et al., 2009; Ursu et al., 2009).

For G types (known as “G types” for “glycoprotein”), the serotypes and genotypes are generally concordant, so G types are usually referred to only by their serotype (e.g., G1). A lack of readily available typing serum or monoclonal antibodies to different VP4 types, however, has hampered the classification of VP4 (or “P types” for “protease-sensitive protein”). Instead, the properties of VP4 have been studied primarily by sequence analysis. P types are generally referred to by their genotype number, which is denoted in brackets (e.g., P [8]) (Estes and Kapikian, 2007). The introduction of molecular typing methods has enhanced our understanding of the diversity of HRV strains, which affects the development of rotavirus vaccines.

MATERIALS AND METHODS

From November 2011 to June 2012, a total of 250 stool samples were collected from children ranging from 1 months to 5 years of age, whom consulted Bint Al-Huda teaching hospital in the province of Nassyriah, Iraq, and suffering from acute gastroenteritis. The children selected in our study had sought medical assistance after several episodes of loose or watery stools, in some cases associated with vomiting. The stool samples were collected using wide-mouthed sterile plastic containers and stored at -20 C until the time of assays. All samples underwent only one cycle of thawing and freezing prior to characterization. A questionnaire were performed to collect data for all patients.

Enzyme-linked immunosorbent assay (ELISA) kits (R-BIOFARM, Germany), were used for screening HRV infections among all stool samples, based on using monoclonal antibodies against the sixth viral gene (VP6). A 96 HRV- Negative stool samples were screened by Real time - quantitative Polymerase Chain Reaction (RT-qPCR) technique by using the primers and TaqMan probe specific for non-structural protein 4 gene (NSP4) and according to the instructions of the manufacturer company (Accupower®Rocket Script TM RT-qPCR, Bioneer, Korea). Statistical analysis was carried out using Chi-square (χ^2) test to evaluate results. P values of less than 0.05 and 0.01 considered to be statistically significant.

RESULTS AND DISCUSSIONS

Out of 250 stool samples from children with acute gastroenteritis, which screened by ELISA and RT-PCR techniques, there were 72 rotavirus-positive (28.8%). ELISA test results showed that there were 54 RV-positive sera with a percentage of (21.6%)

Diarrhea is leading cause of death in children under five years of age globally, with an estimated 1.5 million child deaths per year (WHO, 2008). Rotavirus infection remains the common cause of severe dehydrating diarrhea among children worldwide (Parashar et al., 2006). Each year rotavirus causes an estimated one million episode of diarrhea requiring only home care, 2 million hospitalization and 400,000 deaths in children under 5 years, 82% of which occurs in the poorest countries (Binka et al., 2003; Parashar et al., 2009). Children in developing countries die more because of several factors including poorer access to dehydration therapy and greater prevalence of malnutrition (Nakawesi et al., 2010).

RT-qPCR technique was used to detect the presence of rotavirus in 96-negative ELISA samples. The results showed that there were 18 stool samples showed RV-positive reaction for NSP4 gene with a percentage of infection as (18.75%). The sensitive of PCR- based methods is 100,000 more than that of PAGE. Accurate information on the burden of rotavirus gastroenteritis are crucial to guide recommendation for rotavirus vaccination (Van Damme et al., 2006). Most epidemiological data have been based in survey studies data, thus, as the distribution of circulating rotavirus serotypes may vary dynamically, prospective studies are needed to help the health authorities in planning effective immunization strategies, and for new up-to-date rotavirus vaccines (Zuccotti et al., 2010). In the present study, RT-qPCR technique was conducted by targeting the non-structural protein-4 gene (NSP4) as a universal gene to detect the presence of RV infection. The mechanism of rotavirus induced diarrhea is not fully understood, but it is caused in parts by the first described viral enterotoxin-rotavirus non structural protein 4 (NSP4) (Eset et al., 2001). NSP4 has pleiotropic properties in cells related to its involvement in both rotavirus pathogenesis and morphogenesis (Ball et al., 2005).

All of RV- positive children were suffered from diarrhea (100%), in addition to vomiting and fever with 31(43.05%) and 15(20.83%), respectively. A sudden onset of symptoms typically manifests in children 1 to 2 days after infection with RV. The clinical picture of RV- gastroenteritis is characterized by 4 to 7 days of acute febrile illness, vomiting, and watery, non bloody diarrhea. This combination can lead to rapid dehydration without appropriate intervention. Secondary infection with RV are clinically milder or asymptomatic (Anderson and weber, 2004). Compared with other viral enteropathogens, rotavirus is more likely to result in symptomatic illness and lead to dehydration, electrolyte imbalance, and even convulsion in severely ill patient (Oryan et al., 2009; Yang et al., 2010). The present study results showed that diarrhea was the predominate symptom among RV-infected children included in the study. These results was slightly, different from what Al-Jabiry (2009) found in Thi-Qar province with an incidence of vomiting and diarrhea as RV symptoms with 60% and 40%, respectively. On other hand, our finding was similar to that found in Iran recently (Karager et al., 2012).

In Iraq the death rate in children under 5 years of age was reported to be 130/1,000 males and 120 /1,000 for females in 2003 (WHO, 2003). The results of the present study showed a lower RV seropositive percentages comparing to other similar local studies that used the same serological diagnostic tool (commercial ELISA kits) as in the studies of Hussein and Hassan (2000) in Basrah, and Ahmed et al. (2006), in Erbil (Kurdistan), and Al-khafaji and Al-Jiboury (2013) in Babylon, with RV- positive cases of 43.3% and 37% and 45.76%, respectively.

Males were tend to be more effected by RV with 40 (55.56%) cases in compares to females with 32 (44.44%) cases (figure 1). Statistically, gender differences were non-significant ($p>0.05$). These male predominate in the present study was in agreement with the results of some local studies (Hussein and Hassan, 2000; Al- Jabiry, 2009; Ali *et al.*, 2010), and some regional neighboring countries (Kargar *et al.*, 2012 ; Al –Musawi *et al.*, 2013). Some studies had found a relation between the risk of RV diarrhea and male gender in addition to other factors such as, poor food highgiene, education level, low birth weight, maternal smoking and other (De wit *et al.*, 2000 ; Dennehy, 2006 ; Albano *et al.*, 2007).

The age groups of (1-7) and (8-15) months were the groups that showed, significantly, the most RV - positive infection with a total of 64 (88.89%) cases (figure 1), distributed to 34 (47.22%) and 30 (41.67%) for both age groups, respectively. On the other hand the age group of (16 - 24) month showed the fewest of RV - positive infections with 8 (11.11%) ($p < 0.01$). Rotavirus disease is most common and severe in children between 3- 36 months of age (Bernstein and ward, 2004; yang et al., 2010) the results of the present study according to age distribution were similar to other survey studies in Iraq (Hussein and Hassan, 2000; Ali et al., 2010).

The incidence of RV diarrhea in relation to residence showed different proportion of patients living in the urban area with 43 (59.72%) patient, while the rural area patients recorded less incidence with 29 (40.28%). This variance was statically non significant ($p > 0.05$). The slight increasing of RV-incidents in urban area can be to some extent be explained by the crowded of population in compares to that of rural area. During the study period, April recorded the highest number of RV infected children with 27 (37.50%) cases ($p < 0.01$), followed by December and May with 9 cases (12.50%) for both (figure 2). As unexpected, January showed the lowest RV-positive diarrhea cases with 2 (3.70%), because Rotavirus gastroenteritis usually peaked in winter and early spring (Zuccotti et al., 2010).

The result of the present study seems to be similar to the finding of Al-Musawi et al. (2013) in kingdom of Bahrain. On the other hand, a lot of argument revolves around the credibility of a this subject began to take another turn after the results of some recent studies which recorded high RV- positive infections in summer months compared to winter (Kargar et al., 2012). Our present data demonstrated that there was significant correlation between the seasonal distribution and rotavirus positive cases, during the study period, rotavirus gastroenteritis occurred throughout the year, with more cases occurring in April and May.

The results of the present study showed that the rate of RV gastroenteritis was found to be the highest in children who were used breast feeding with 36 (50.0%) child, followed by bottle feeding and mixed feeding with 25 (34.72%) and 11 (15.28%). This variation of the above results was statistically significant ($p < 0.01$). Other similar study found a different results with a pre dominate RV- positive infection in children with Bottle feeding (Ali et al., 2010). On the other hand, other studies showed similar infection distribution according to the feeding type (Nakawesi et al., 2010).

The present study results showed that breast feeding children tend to be more susceptible to face RV diarrhea. Breast feeding has consistently been showed to confer a protection against non - viral gastroenteritis pathogen, but evidence for viral protection has remained weak (Golding et al., 1997). It is possible that breast feeding may only be protective if it is practiced with an intensity and frequency that allows continues high level protection of the intestinal mucosa rather than sporadic or low volume feeds (Glass et al., 1986).

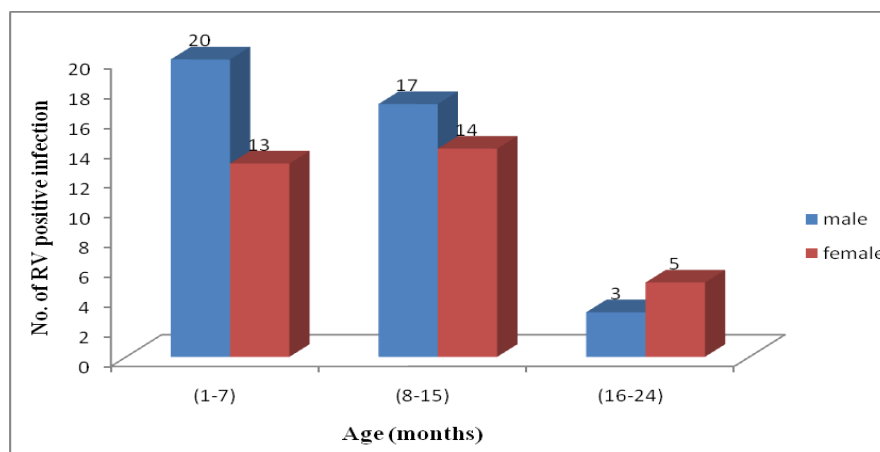


Figure 1: Distribution of RV Positive Infection According to Age and Sex

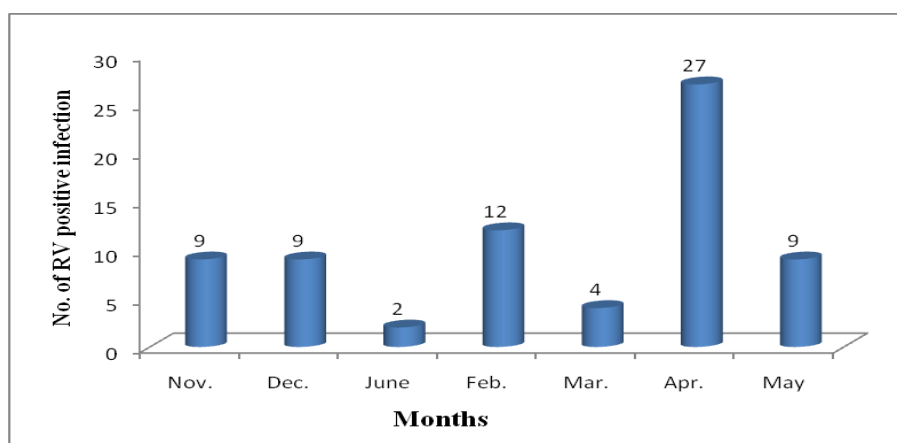


Figure 2: The Distribution of RV Positive Infection According to the Study Period

CONCLUSIONS

The purpose of the current study to detect the spread of disease within the rotor filtrate children aged less than 5 years and who reviewed the hospital girl Huda education in the province of Dhi Qar, Iraq. The study included 250 stool samples collected from children suffering from acute diarrhea were conducted to detect infection Balrashh rotor using a technique adsorption-linked immunosorbent assay (ELISA) as checking immunological testing chain reaction amorphous quality - real-time (RT-qPCR) as a technique vital during the period from October the second month of 2011 to June 2012.

Total of 250 stool samples, the number of injuries Balrashh rotor 72 (28.8 %) and using both methods. The results of the ELISA assay revealing positive for RV with 54 cases (21.06 %). Test was used for qPCR RT- detect the presence of leachate rotor RV out in 96 sample showed a negative account of the ELISA test. The results showed the presence of 18 samples out had revealed positive toward gene NSP4 and injury rate was (18.75 %). The most visible clinical symptoms are diarrhea 72 (100%) in addition to vomiting, with 31 (43.06 %), fever in 15 (20.83 %).

The males are more susceptible to Balrashh rotor and the 40 children (55.56 %) compared with females living with a child with 32 (44, 44%) $P > 0.05$), showed age groups (1-7 months) and (8-15 months) the highest number of injuries to 64 children (88, 89%) $p < 0.01$). Within the study period, record the month of April and the moral behind the largest number of injuries Balrashh rotor with 27 (37.50 %) Baby $p < 0.01$) on the other hand showed the month of January the lowest number of injuries only two cases (3.70 %).

The results of the present study was that the highest incidence of inflammation of the intestines caused by leachate rotor has emerged in patients who use breastfeeding to 36 children (50.0 %) followed by children who use artificial feeding and mixed with 25 (34.72 %) and 11 (15.28 %) children , respectively, $p < 0.01$). Showed patients who consume Balrashh rotor type RO drinking water and a difference of moral higher proportion of injuries to 36 children (50.0 %) compared with children who use the mineral water or tap water which record the 23 (31.94 %) and 13 (18 , 06 %) children , respectively, $p < 0.01$).

Given the previous results it can be concluded that the incidence of dizziness in perpetuating Balrashh cases of acute diarrhea, may be common in the province of Dhi Qar, and perhaps getting a sustained manner.

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